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ORIGINAL PAPER

Physical and Sensory Attributes of a *trans*-Free Spread Formulated with a Blend Containing a Structured Lipid, Palm Mid-Fraction, and Cottonseed Oil

Stephen E. Lumor · Ashanty M. Pina-Rodriguez · Robert L. Shewfelt · Casimir C. Akoh

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Abstract Physical and sensory attributes of an experimental *trans*-free margarine spread (MG-X) and two commercial margarine spreads (MG-A and MG-B) were studied. The *trans*-free margarine spread was formulated with a blend containing a structured lipid (SL) synthesized by reacting canola oil with 40% stearic acid (w/w), palm mid-fraction (PMF), and cottonseed oil (CTO). No *trans* fatty acids were detected in MG-X, whereas the *trans* fatty acid contents of MG-A and MG-B were 0.3 and 3.7% (w/w), respectively. MG-X was considerably firmer than MG-A and MG-B, less cohesive, and its adhesiveness was intermediate between those of MG-A and MG-B. MG-X's stability to syneresis was also intermediate between those of MG-A and MG-B. Sensory evaluation showed that MG-X was comparable to MG-A in terms of spreadability and texture only, but was significantly different from MG-B in all attributes.

Keywords Canola oil · Cottonseed oil · Palm mid-fraction · Stearic acid · Structured lipid · *trans*-Free margarine spread

Introduction

Consumption of significant amounts of *trans* fatty acids (TFA) has been a major health concern for consumers and regulatory agencies over the past decade. The major dietary sources of *trans* fatty acids are products formulated with partially hydrogenated fats. Examples include margarines, shortenings, bakery products, and fast foods. The amount of TFA present in these foods is estimated to be between 0 and 35% of total fatty acids [1], and the mean daily intake of TFA per person in the US population is 2.6% energy [2]. Intake of high amounts of TFA has been correlated with increased risk for cardiovascular diseases, primarily due to their adverse effects on plasma lipid profile [3, 4]. These health concerns have led to a near global regulation of the levels of TFA in food. In the United States, the Food and Drug Administration (FDA) issued a final ruling requiring foods containing TFA to be labeled accordingly, effective from January 2006 [5]. This regulatory mandate and consumer concerns have led to the development of alternative processes that will produce foods with zero or reduced TFA contents. The synthesis of structured lipids (SL) by the process of transesterification is one of such alternatives. This process does not result in the production of TFA, as is the case with partial hydrogenation [1].

The aim of this study was to evaluate physical and sensory attributes of a *trans*-free margarine spread produced from a blend of a structured lipid (synthesized by enzymatically incorporating stearic acid into canola oil), with palm mid-fraction (PMF) and cottonseed oil (CTO). PMF was added to increase solid fat content, and together with CTO, promote β' crystal formation to impart smooth texture to the margarine spread. This study is of significance because death from degenerative diseases such as cancer, cardiovascular disease, obesity and diabetes can be reduced significantly by adhering to healthy diets. The production of margarine spread with zero *trans* fat content will therefore be beneficial.

S. E. Lumor · A. M. Pina-Rodriguez · R. L. Shewfelt · C. C. Akoh (✉)
Department of Food Science and Technology, University of Georgia, Athens, GA 30602-7610, USA
e-mail: cakoh@uga.edu

S. E. Lumor
e-mail: enyam1@gmail.com

Materials and Methods

Materials

Stearic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Canola oil (peroxide value 0.0 mequiv/Kg, acid value 0.28%) was purchased from a local grocery store. Palm mid-fraction (peroxide value 1.0 mequiv/Kg, acid value 0.1%, iodine value 43–48) was donated by Fuji Vegetable Oil Inc. (Savannah, Georgia), and cottonseed oil (peroxide value 0.0 mequiv/Kg, acid value 0.15%) by Archer Daniels Midland Co. (Valdosta, Georgia). Immobilized Lipozyme RM IM was purchased from Novo Nordisk A/S (Bagsværd, Denmark). Sorbitan tristearate (STS) with hydrophile/lipophile balance (HLB) of 2.1, and distilled monoglyceride (DMG) with HLB of 3.8 was donated by Danisco A/S (Copenhagen, Denmark), Annatto OS 15 food color was donated by D. D. Williamson Colors, LLC (Port Washington, WI, USA). ButterBuds Food Ingredients (Racine, WI, USA) supplied margarine flavors. Organic solvents and chemicals were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) or Fisher Scientific (Norcross, GA, USA). All other chemicals used were of analytical or HPLC grade.

Structured Lipid Synthesis

Structured lipid synthesis was performed in a stir-batch reactor at 65 °C for 12 h. The reaction mixture typically contained 300 g of canola oil, 40% stearic acid (by weight of canola oil), and 10% Lipozyme RM IM (by total weight of reactants). The product was separated from the enzyme by suction filtration, and free fatty acids removed by short-path distillation with a KDL-4 (UIC Inc., Joliet, IL, USA) unit under the following conditions: heating oil temperature, 185 °C; cooling water temperature, 55 °C; pump vacuum, <1 mm Hg; feed rate, maintained at 100 mL/h. The reaction product was passed through the system twice to reduce the free fatty acid content. Free fatty acid content of samples (0.12–1.05% oleic acid) was determined according to AOCS Official Method, Ca 5a-40 [6].

Margarine Spread Production

Formulation of the spread was based on a previous study [7] in which the physical and crystal properties of a blend of a structured lipid (SL40) and palm mid-fraction (PMF) were evaluated. SL40 was blended with PMF and cottonseed oil (CTO) in the ratio of 11:7:3 (w/w/w). The compositions of the lipid and water phases are given in Table 1. The lipid phase (70%, w/w) was kept at 80 °C for 1 h to melt components, after which the aqueous phase (30%, w/w) was added and the mixture homogenized at 1,000 rpm for 5 min using a Gifford-Wood's Rotor–Stator Homogenizer Model 2L (Hudson, NH, USA). The emulsion was crystallized in a batch ice cream freezer model 104 (The Taylor Company, Rockton, IL, USA) for 10 min. The output temperature was −10 °C. The product was tempered at room temperature (22 °C) for 2 h, after which it was worked with a hand whip for 10 min. The product was then poured into containers and stored at 0 °C. For comparison, two commercial margarine spreads (MG-A and MG-B) were melted and crystallized as described above.

Table 1 Formula for experimental margarine spread (MG-X)

Ingredient	wt%
Lipid phase	
SL40:PMF:CTO (5.5:3.5:1.5. w/w)	67.17
Lecithin	0.20
DMG:STS (3:2, w/w)	0.50
Tenox 20A antioxidant	0.10
Annatto food color	0.03
Butter buds (flavor)	0.50
Aqueous phase	
Water	27.25
Skim milk powder	1.25
Salt	1.50
Buttermilk buds (flavor)	1.50

SL40 structured lipid made by reacting canola oil with 40% stearic acid, *PMF* palm mid-fraction, *CTO* cottonseed oil, *DMG* distilled monoglycerides, *STS* sorbitan tristearate

Fatty Acid Profile Analysis

Between 0.1 and 0.2 g of fat (in duplicate) from each margarine spread was converted to fatty acid methyl esters (FAME) using AOAC Official method 996.01, Section E [8] as previously described [9]. The FAME were analyzed in parallel with a FAME standard (Supelco 37 component FAME mix, Supelco®, Bellefonte, PA, USA) using an Agilent Technology 6890N gas chromatograph equipped with a flame-ionization detector (FID). An SP-2560, 100 m × 0.25 mm ID, 0.20 μm film column was used for separation. One injection (1 μL) per sample in duplicate was performed in the split mode, at a split ratio of 50:1. Helium was the carrier gas, the linear velocity was 20 cm/s, and the flow rate was 1 mL/min. The oven temperature was initially held at 140 °C for 5 min and then programmed to 240 °C at 4 °C/min, and held isothermally for 20 min. The injection port temperature was 250 °C while that of the detector was 260 °C. The different amounts of FAME were analyzed and integrated by an online computer, and values for duplicate samples averaged to give fatty acid profile of each sample (Table 2).

Table 2 Fatty acid profile of experimental (MG-X) and commercial (MG-A, MG-B) margarine spreads

Fatty acid	MG-X	MG-A	MG-B
C16:0	17.2 ± 0.3	26.0 ± 0.2	10.4 ± 0.2
C18:0	14.9 ± 0.0	4.3 ± 0.0	12.5 ± 0.2
C18:1 *cis*	40.4 ± 0.2	37.2 ± 0.1	24.5 ± 1.2
C18:1 *trans*		0.3 ± 0.0	3.2 ± 0.1
C18:2 *cis*	22.7 ± 0.2	25.9 ± 0.2	40.9 ± 0.7
C18:2 *trans*			0.5 ± 0.0
C18:3 ω6	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
C18:3 ω3	2.7 ± 0.0	3.2 ± 0.0	6.1 ± 0.1
C20:0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
C20:1 ω9	0.9 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
Others[a]	0.7 ± 0.0	2.3 ± 0.0	1.9 ± 0.0
ω6:ω3	8.5	8.1	6.7
Saturated fat	32.9 ± 0.2	32.4 ± 0.4	24.0 ± 0.2
Unsaturated fat	62.1 ± 0.2	67.6 ± 0.4	75.9 ± 0.2
% *trans* fat		0.3 ± 0.0	3.7 ± 0.1

[a] Sum of C12:0, C14:0, C16:1, C20:0, C21:0, C20:2, C22:0, C20:3 ω6, and C22:1 ω9

Texture Profile Analysis (TPA)

Textural properties (hardness, adhesiveness, and cohesiveness) of margarine spreads were evaluated at 22 °C using the TPA procedure [10]. A double compression test was performed using a TA-X2 texture analyzer (Stable Micro Systems, London, United Kingdom). A 45° conical probe attached to a 5 kg compression load cell was used to penetrate the samples at 1.0 mm/s to a depth of 10 mm from the sample surface, and withdrawn at the same speed. The maximum force (N) during the first compression was reported as hardness. The negative force area in N s (A_2) for the first compression was reported as adhesiveness. The ratio of the positive force area during the second compression (A_3) to that of the first compression (A_1) was reported as cohesiveness. Each sample was analyzed in triplicate.

Rheological Properties

A series of tests were performed on a dynamic stress-controlled rheometer SR5000 (Rheometric Scientific, Piscataway, NJ, USA) at 22 °C to determine the rheological properties of margarine spreads. A 25 mm parallel plate (0.6 mm gap) was used for analysis. In creep analysis, a constant low stress (0.4 KPa) was applied to samples and deformation per unit time measured as an indicator of emulsion stability. The lower the degree of deformation, the less likely the spread will show syneresis [11]. In dynamic analysis, spread samples were subjected to a sinusoidally varying stress (0.4 MPa) and the strain output and phase difference between the input and output signals were measured. Storage/elastic (G') and loss/viscous (G'') moduli were calculated from this information. The frequency (Hz) at which G' and G'' crossover is a measure of spreadability. The higher the crossover frequency, the more spreadable the sample is [11].

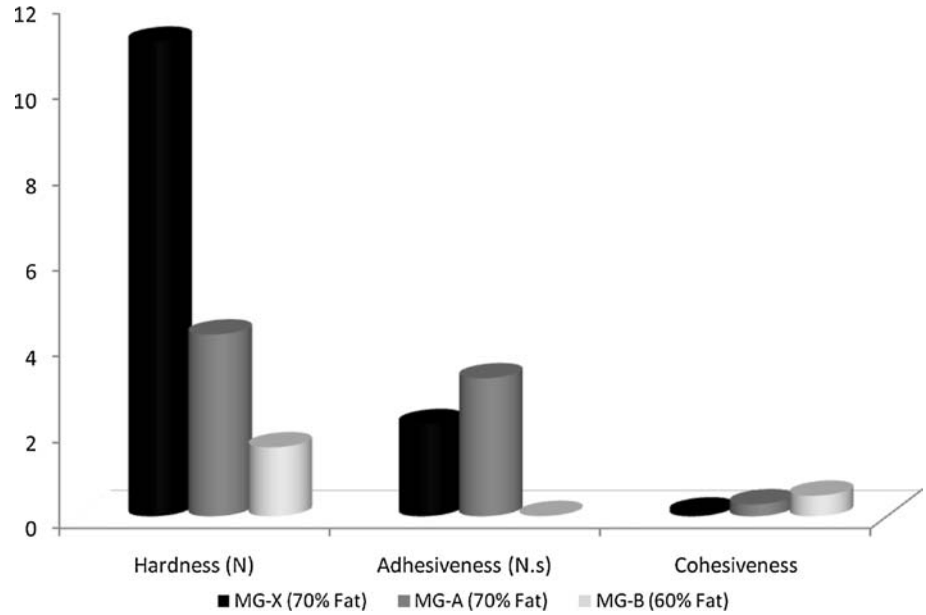
Sensory Evaluation

Sensory evaluation was performed with panelists in individual booths under fluorescent light. Subjects were students/staff of the University of Georgia (UGA). Coded spread samples were presented simultaneously to the subjects together with slices of bread, non-salted crackers, and water. Evaluation was performed by spreading the samples on slices of bread. A ranking (difference) test using 37 untrained panelists was used to rank the margarine products in terms of the attributes, appearance, spreadability, and texture (mouthfeel). No ties were allowed. The average responses for the ranking test were calculated and analysis of variance (ANOVA) performed by means of SAS Statistical Software Version 9.1 (SAS Institute, Cary, NC, USA). Significant differences between samples in terms of each attribute were determined using the Duncan's multiple comparison test.

Results and Discussion

The fatty acid profiles of the experimental margarine spread (MG-X) and commercial margarine spreads, MG-A (70% fat, w/w) and MG-B (60% fat, w/w), are given in Table 2. No *trans* fatty acids (TFA) were detected in MG-X, whereas C18:1t and C18:2t were detected in MG-B, and C18:1t in MG-A. Even though the levels of TFA detected in the two commercial margarine spreads would amount to less than the 4.6% energy as TFA required to adversely affect plasma lipoprotein levels [1], a product containing absolutely zero TFA content would be more desirable. Among the three margarine spreads, MG-A and MG-B contained the lowest levels of C18:0 and C16:0, respectively. The saturated fatty acid (SFA) contents of MG-X and MG-A were comparable. While majority of SFA was C16:0 in the case of MG-A, the contents of C16:0 and C18:0 were about equal in MG-X and MG-B. This is of significance because unlike C16:0, stearic acid contributes to plasticity without having adverse effects on plasma lipoprotein levels [12–15], and can therefore substitute for a fraction of C16:0. This cannot be a full substitution because the level of C16:0 in a fat is critical for the preponderance of β' triacylglycerol (TAG) crystals [16], which impart smooth mouthfeel to margarine.

Fig. 1 Texture profile analysis of experimental (MG-X) and commercial (MG-A and MG-B) margarine spreads. The spreads differed significantly (p value <0.001 at $\alpha_{0.05}$) from one another with respect to the three attributes



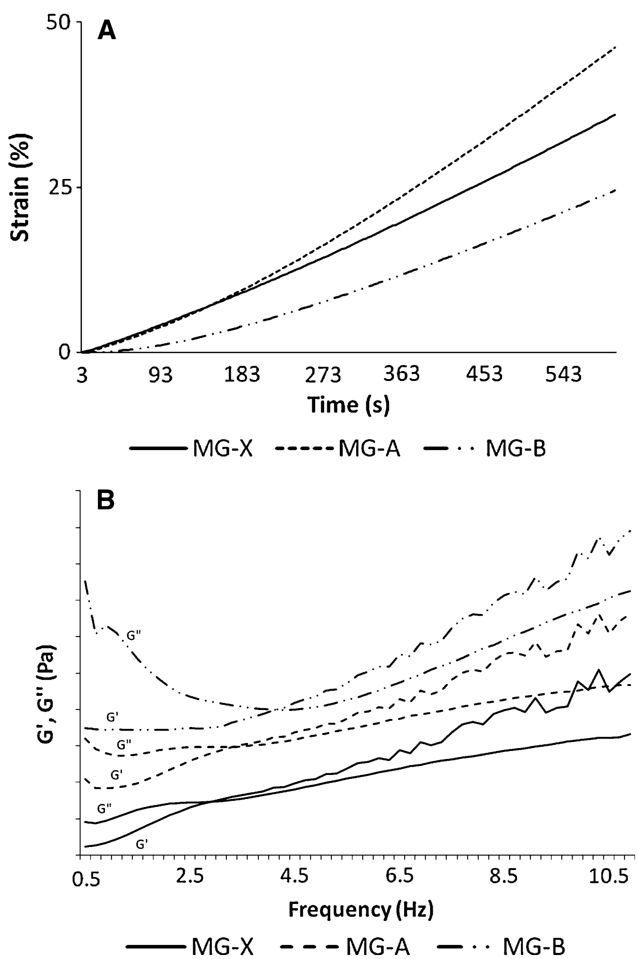


Fig. 2 Creep analysis (**a**) and dynamic analysis (**b**) of experimental (MG-X) and commercial (MG-A and MG-B) margarine spreads

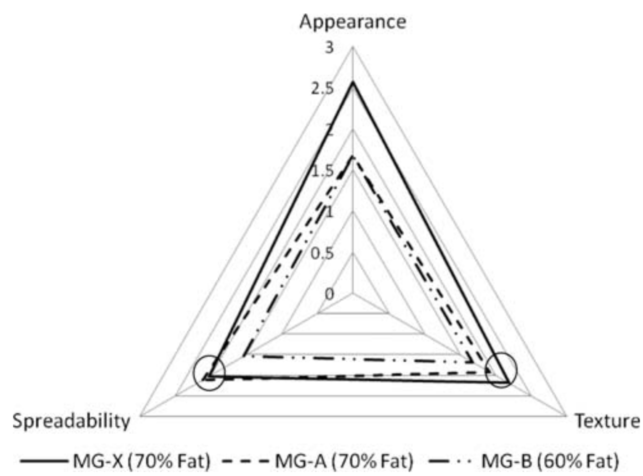


Fig. 3 Mean ranking of attributes of experimental (MG-X) and commercial (MG-A and MG-B) margarine spreads in the ranking (difference) test. The *ovals* represent the points where no significant difference was observed between MG-X and the other spreads. Rank 1 is highest and 3 is lowest

The textural properties (hardness, adhesiveness and cohesiveness) of the margarine spreads are given in Fig. 1. All three spreads were significantly different in terms of each attribute. MG-X had the highest hardness value (N) and will therefore be more difficult to spread compared to the other two. MG-B had the lowest hardness value most probably because it was formulated with less fat (60%, w/w) and the content of SFA was 24% compared to 32.9 and 32.4% for MG-X and MG-A, respectively. Even though MG-X and MG-A contained comparable amounts of SFA (Table 2) and total fat (70%, w/w), the disparity in their hardness values can be attributed to their different TAG compositions. Lumor et al. [9] observed that solid fat content or hardness was mostly dependent on how saturated the TAGs present in the fat or oil were. For two fats having comparable SFA contents, the one having higher levels of highly saturated TAGs will most likely be harder than the one having lower levels of highly saturated TAGs. Solid fat content or hardness can also be affected by the presence of minor components such as emulsifiers. These emulsifiers, together with the types of TAGs present, can influence crystal properties (polymorphism and morphology) and solid fat content, which in turn impact rheological properties of margarines [17]. Adhesiveness (N s) of MG-X was intermediate between those of MG-A and MG-B. MG-B was the least adhesive and most cohesive. MG-X was the least cohesive while MG-A was the most adhesive. The more adhesive a spread is, the more likely it will stick to utensil such as spreading knife. Cohesiveness is a measure of intermolecular strength. The spreadability of margarine is affected to different extents by adhesiveness and cohesiveness.

Figure 2 shows the rheological properties of the margarine spreads. Creep analysis (Fig. 2a) is a measure of emulsion stability. A low constant stress (0.4 MPa) was applied to samples and deformation (strain) per unit time measured. The sample that showed the least deformation with time would be the least likely to show syneresis. The order of spread emulsion stability was MG-B > MG-X > MG-A. In dynamic analysis (Fig. 2b), a sinusoidally varying stress (0.4 MPa) was applied to samples. Storage/elastic (G') and loss/viscous (G'') moduli were calculated and plotted as a function of frequency (Hz). The frequency at which G' and G'' crossover was indicative of spreadability. The higher the crossover frequency, the more spreadable the sample was. The order of spreadability was MG-B > MG-A > MG-X.

Sensory evaluation is an important tool that links product attributes with consumer preferences. Figure 3 shows results of the ranking test of the experimental margarine spread (MG-X) and the two re-crystallized commercial margarine spreads (MG-A and MG-B). Subjects actively differentiated between samples by ranking them (rank 1 was highest and rank 3 was lowest), and no ties were allowed. MG-A and MG-B were not significantly different with respect to all attributes except for spreadability where MG-B had a higher mean ranking. MG-A was not significantly different from MG-X in terms of spreadability and texture whereas MG-B was significantly different. Our results also show perfect agreement between dynamic analysis (Fig. 2b) and sensory analysis (Fig. 3) with respect to spreadability. In both cases, MG-X and MG-A were closer in terms of spreadability compared to MG-B, and the order of spreadability was MG-B > MG-A > MG-X.

Conclusions

Foods with zero or reduced *trans* fat are becoming more desirable because of health implications associated with significant consumption of *trans* fatty acids. The experimental margarine (MG-X) formulated in this study did not contain *trans* fatty acids and contained less palmitic acid compared to MG-A. Sensory evaluation showed that MG-X was not significantly different from MG-A with respect to spreadability and texture, but was significantly different from MG-B in all attributes.

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